DESIGN, SYNTHESIS, CHARACTERIZATION, ANTI-TUBERCULAR ACTIVITY AND IN SILICO STUDIES OF NOVEL THIOPHENE ANALOGUES

SYNOPSIS OF THE THESIS

Submitted by

Priyankul Palia

Registration No. AU20PYPhD0042

Under supervision of

Dr. AMIT CHAUDHARY

In Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

SCHOOL OF PHARMACY

ABHILASHI UNIVERSITY

CHAIL-CHOWK, MANDI (H.P.) – 175028

JANUARY-2022

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DECLARATION

I hereby declare that the dissertation synopsis entitled "**Design, Synthesis, Characterization, Anti-Tubercular activity and In silico studies of novel Thiophene analogues**" to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

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1. INTRODUCTION

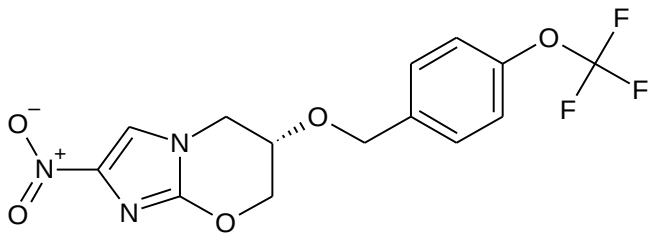
Tuberculosis, an ancient disease, that spreads through air, most contagious caused by Mycobacterium tuberculosis. It is one of the leading causes of death among the top ten diseases worldwide. About 10 million new cases and 1.6 million were died in the year 2017[1]. Out of which 8 percent were HIV positive. Nearly 16-20% of the previously treated cases and 3.55 of new cases were found to have MDR-TB [2][3].

Multidrug resistance and extensively drug resistance hamper efforts to decrease the burden of TB. Patients like HIV positives cases were probably co-infected with TB become more life threatening. Moreover, treatment relapse becomes the vulnerable reason behind drug resistance makes the scenario complicated. The poor patient compliance and complex treatment regimens, longer duration of therapy anticipates to the pivotal cause for drug resistance [4]. From 1998, on complete exploration of the mycobacterium genome sequences and deeper insights in to the epidemiology of TB, the treatment for the disease becomes a really a tough task. In view of tuberculosis treatment at different concepts like existing tb drugs which are less potent, co-morbidity, poor patient non-compliance, unusual treatment response in clinical trials from currently available molecules. From all this situation it is distinctly clear that, there is an urgent need for discovering newer anti-tuberculosis agents which are effective in drug resistance and resistant strains.

From the past decade, new chemical entities were introduced which are still under clinical trials. Out of which two drugs were in conditional regulatory approval as they have potential side effects and black box warning. Drug repurposing is another strategy for the exploration of research in Tb. Seven repurpose drugs have also moved ahead for testing under tb pipeline. There are seven new combinations of drugs have been tested in Phase II and Phase III clinical trials. From all these findings and efforts, research reports that are still not convincing regarding the drug resistance which enforces the emergence of developing novel anti-tubercular agents. Thiophene based compounds have been reported to possess antimicrobial, analgesic, anti-inflammatory, antihypertensive and antitumor activity [5-7]

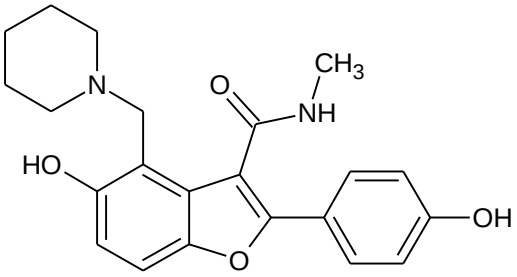
2. LITERATURE REVIEW

Andrew M.Thompson et al (2017) identified a new preclinical moiety from pretomanid. 7-substituted imidazoxazines plays a very important heterocycle in the field of research in tuberculosis. PA-824 pretomanid acts on inhibition of mycolic acid biosynthesis (blockage of oxidation of hydroxymycolate to ketomycolate) possessing imidazo oxazine moiety. They acts against replicating and non-replicating hypoxia adapted bacilli. It exhibits its potential extremely on drug sensitive and multi drug resistant tuberculosis with the MIC of 0.015 – 0.250 µg/ml.[8]



PA-824 Pretomanid

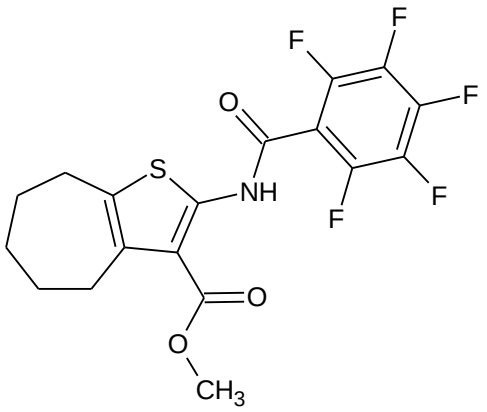
Aggarwal et al., (2017) identified a new lead from structure guided medicinal chemistry, TAM16, is a benzofuran class inhibitor of Pks13 with highly potent in vitro bactericidal activity against drug-susceptible and drug-resistant clinical isolates of M. tuberculosis with the MIC of 0.09 µM.[9]



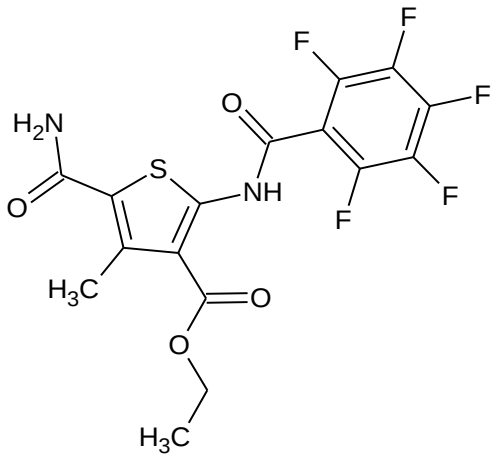
TAM 16

Regina Wilson et al (2013) synthesized a series of antitubercular thiophenes which acts on M.tuberculosis by an uncharacterized mechanism of action showing PKS13 inhibition in

mycolic acid biosynthesis.[10] Two compounds namely TP2 and TP4 showed excellent sterilizing action on M.tb and drug resistant strains. However TP4 shows higher activity at 0.5 µM than TP2 at 1.0 µM

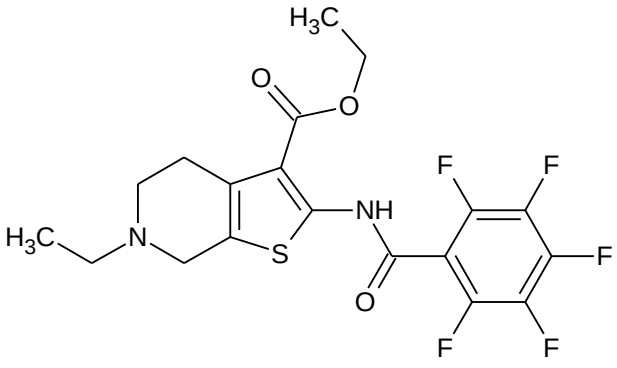


(TP2)



(TP4)

Sandeep Thanna et al (2013) synthesized a series of 2-amino – 4,5,6,7- tetrahydro thieno [2,3-c] pyridines against sensitive and drug resistant Mycobacterium tuberculosis with MIC of 0.69 µM by MABA method.[11]



Patent No.	Title
WO2003084947A1	4,5,6,7-tretrahydrobenzo[b] thiophene derivatives and methods for medical intervention against mycrobacterial infections
US9447082B2	Optically active fluconazole analogues containing thiophenes as antifungal agents
US4740519A	Benzo (b) thiophene carboxylate derivatives and pharmaceutical compositions

RESEARCH GAP IN THE WORK AND PROBLEM DESCRIPTION

Tuberculosis, a major contagious air borne disease, usually kills millions to billions in a year. It is considered as one of the top ten diseases among worldwide. Many of the existing anti-tubercular drugs suffers from resistance as they do not have potential ability to overcome this resistance phenomena. They are highly bactericidal but later on due to several factors like poor patient compliance, incomplete therapy etc., leads to treatment relapse. In order to execute the situation, the current trends in drug discovery process eventually utilize CADD. It plays a crucial role in drug development. So, the work proposed is to track the novel leads against tuberculosis were carried out in two different methods namely LBDD and SBDD. The first approach in LBDD is QSAR. To interpret the relationship of a molecule and physicochemical attributes to biological activity. Further, best compounds were computationally evaluated at different levels like docking and molecular dynamics simulation. The other approach is SBDD, is to modify and identify leads from the reported literature. Best compounds were selected for synthesis, based on docking interactions, later evaluated for characterization and anti-tubercular activity by MABA method.

Compounds were developed from the thiophene scaffold representing the MIC of 0.5 -1.0 µg/ml, tested against H37Rv from Regina Wilson et al (2013) and Sandeep Thanna et al (2016). Compounds with 2-Fluoro phenylamide and 4- Fluoro phenylamide shows loss in activity.

3. AIM & OBJECTIVES

AIM

The aim of the present work is to design and develop small bioactive heterocyclic derivatives as anti-tuberculosis agents.

OBJECTIVES

- In-silico studies of proposed compounds
- To synthesize novel thiophene derivatives
- Structural characterization of synthesized compounds by instrumental methods of analysis like IR, NMR and Mass spectroscopy.
- In-vitro anti-tuberculosis activity of synthesized compounds.
- In-vivo toxicity and pharmacokinetic studies of synthesized compounds.

4. PROPOSED METHODOLOGY OF RESEARCH WORK

A. Experimental Work :

4.1 Design of Thiophene based compounds: POTENT TARGET – PKS13

The organism mycobacterium possesses a unique cell wall structure. Its structural composition is of various components in different proportions. Of which mycolic acid lipid layer found to be the major contributor in making up the cell wall. Mycolic acids are very long chain fatty acids synthesized by the enzymes called FA synthase I and Fatty acid synthase II complexes. Out of these two, PKS 13 lies on the peripheral where it condenses the mycolic acids synthesized by the FAS I & II complexes to form an enormous chain of fatty acid. Drugs which are available for treatment, usually becomes failure as the molecule would not be able to rupture the fatty acid layer and they exhibits resistance. [12] However, on exploration of the whole genome analysis on mycobacterium tuberculosis, complete evaluation for different causes and its epidemiology, and deeper insights into drug resistance, identifying a valid target is crucial and molecules were designed to hit the same will be the key towards tuberculosis drug discovery. Polyketide synthase XIII is found to be the vulnerable target for hitting M.tb. Overall the percentage of resistance was comparatively lower than the other targets [13].

4.1.1 STRUCTURE AND IMPORTANCE OF POTENT TARGET PKS13

Nearly around twenty enzymes are known to be involved in the synthesis of Mycolic acids. Out of which polyketide synthase constitutes an important family of enzymes accounts for mycolic acid condensation. Twenty four different PKS that are linked and different PKS isoforms are available.[7][8][9] From which PKS13 is recognized as a desirable target for tuberculosis treatment. It is usually composed of 1733 amino acids forms around five distinct domains namely acyl carrier, beta keto acyl synthase, acyl transferase and thioesterase functions. All the domains are needed for the condensation reaction. The thioesterase domain serves to catalyze for both the release and acyl transfer reactions that are necessary for the formation of mycolic acid biosynthesis. Therefore the PKS13TE is bestowed as potential TB drug target. [14-16**]**

4.1.2 CADD – QSAR DOCKING AND DYNAMICS

Usually, a combined strategy is always considered to be a reliable tool for drug discovery. In this case, Combination of computational and experimental approach has become comparatively more popular than traditional techniques. Therefore, QSAR, Molecular docking and Molecular dynamics are likely to be the hallmark for antitubercular drug discovery.

The present study deals with the identification of new inhibitors against pks13 under two different approaches. The first being QSAR approach, where the dataset molecules are subjected to find the model equation based on descriptor analysis. Then, molecules will designed and subjected to model equation to attain the expected P values such that the molecules becomes fruitful against the crucial targets. The best leads will further filtered through molecular docking to elucidate better binding interactions and potential energy. Finally the molecules which comes best out of binding energy will selected for ADMET prediction to rule out various toxicities by protox II server.On further investigating the binding mode and stability of inhibitor inside the binding pocket, the top score leads will subjected to molecular dynamics simulation studies and it will analyzed for RMSD, RMSF and secondary structure analysis.

The second approach is ligand based drug design, where the molecules are designed based on the recently reported literature. From which the top activity molecules will be the better source for designing new molecules which acts against PKS13 inhibitor. Further the compounds will scrutinized for good binding affinity through docking. Molecular complexes with potential binding interactions and least energy were subjected to ADMET prediction. The molecules will check for various physicochemical properties and toxicities. Finally the compounds will opt for synthesis and evaluation of antitubercular activity.

4.2 SYNTHESIS :

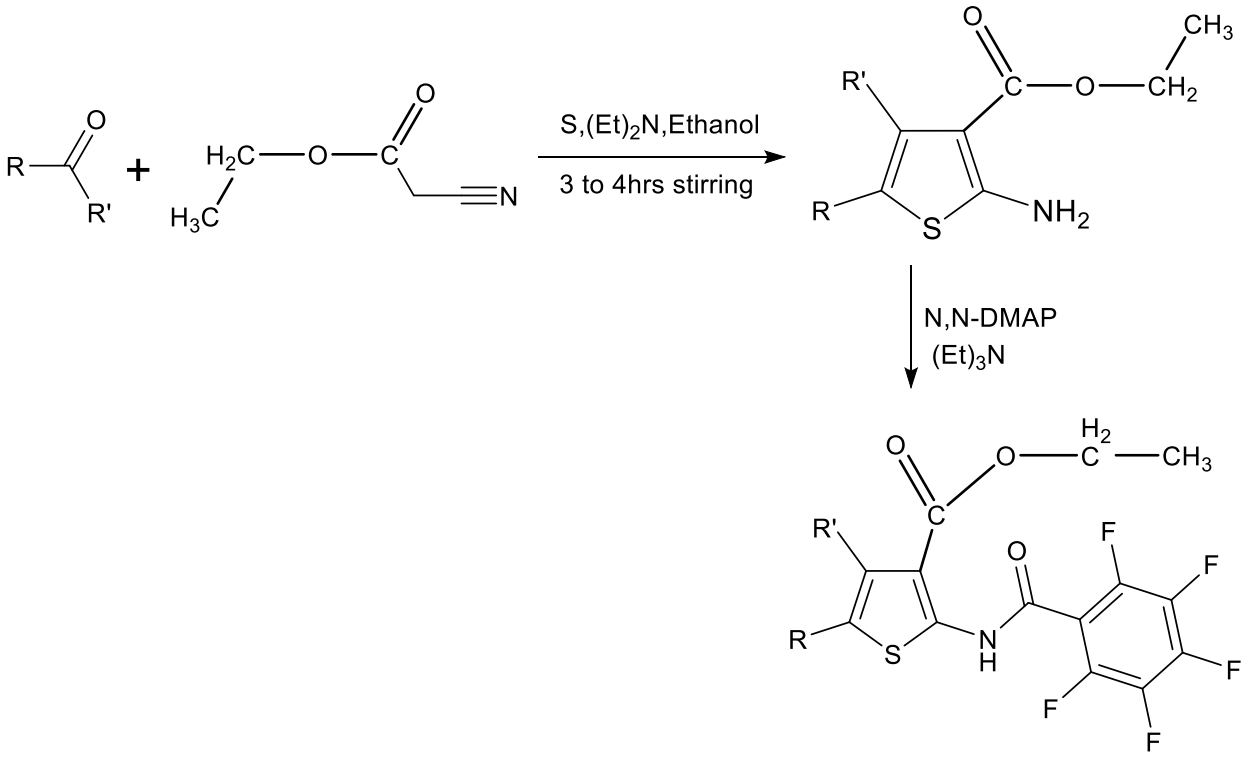
Step I: Synthesis of thiophene 2-amino esters:

To the weighed quantities of 0.1 mole of alpha methylene carbonyl compound, sulphur and alkyl cyano acetate will be dissolved in 20 ml of ethanol. Mixed and stirred together. To this well stirred mixture diethyl amine (0.125 moles) will added drop wise and stirring continued for about 3 hours at ambient temperature.[30] After which the reaction mixture was refrigerated overnight. On the next day the separated solid was filtered and washed with 20 ml of chilled aqueous methanol. The brown solid which obtained will dissolved

in dichloro methane and washed with saturated ammonium chloride 20 ml, deionized water 20 ml and saturated sodium chloride 20 ml. The dichloro methane layer will separated using the separating funnel. To this, anhydrous sodium sulphate will add and filtered. The solvent will evaporated to get the crude product which is then purified by recrystallization from cold methanol to obtain a yellow solid.

Step 2: Synthesis of penta fluoro derivatives from amino esters.

Compound obtained from step 1 will dissolve in dichloro methane and cooled to 0 degrees. N,N-dimethyl amino pyridine and triethyl amine was added. To this pentafluoro benzoyl chloride was added drop wise and allowed to stir for 12 hours at room temperature. After which the solvent was evaporated to dryness to obtain a crude solid. It will then purified by methanol to get pure pentafluoro derivatives.



R,R'=Aryl/cyclic ketones

Figure 1: Synthetic Scheme

4.3 CHARACTERIZATION

The compounds will be characterized by IR, NMR and MASS spectroscopy.

4.4 BIOLOGICAL EVALUATION

Mycobacerium tuberculosis activity will be done by Microplate Alamar Blue assay method against H37Rv.

Isoniazid- 1.6 µg/ml, Ethambutol - 1.6 µg/ml, Pyrazinamide- 3.125µg/ml, Rifampicin - 0.8 µg/ml and Streptomycin- 0.8µg/ml

Procedure : In response to the need for rapid, inexpensive, high-throughput assays for anti-mycobacterial drug screening, a micro plate-based assay which uses Alamar blue reagent for determination of growth was evaluated. MICs of all the synthesized antimicrobial agents against Mycobacterium tuberculosis H37Rv and M. tuberculosis H37Ra will examined in the microplate Alamar blue assay (MABA) with both visual and fluorometric readings and compared to MICs determined in the BACTEC 460 system. For all three mycobacterial strains, there is < or = 1 dilution difference between MABA and BACTEC median MICs in four replicate experiments for most of the antimicrobics. Significant differences between MABA and BACTEC MICs will be observed with some of the antimicrobial agents. Overall, MICs determined either visually or fluorometrically in MABA will highly correlate with those determined in the BACTEC 460 system, and visual MABA and fluorometric MABA MICs will highly correlated. MICs of rifampin, rifabutin, and minocycline were consistently lower for H37Ra compared to H37Rv in all assays but similar for most other drugs. M. tuberculosis H37Ra may be a suitable surrogate for the more virulent H37Rv strain in primary screening of compounds for antituberculosis activity. MABA is sensitive, rapid, inexpensive, and non-radiometric and offers the potential for screening, with or without analytical instrumentation, large numbers of antimicrobial compounds against slow-growing mycobacterium.[17,18,19]

4.5 In Vivo studies of synthesized compounds

4.5.1 Toxicity studies

4.5.2 Pharmacokinetic studies

5. PLAN OF THE WORK

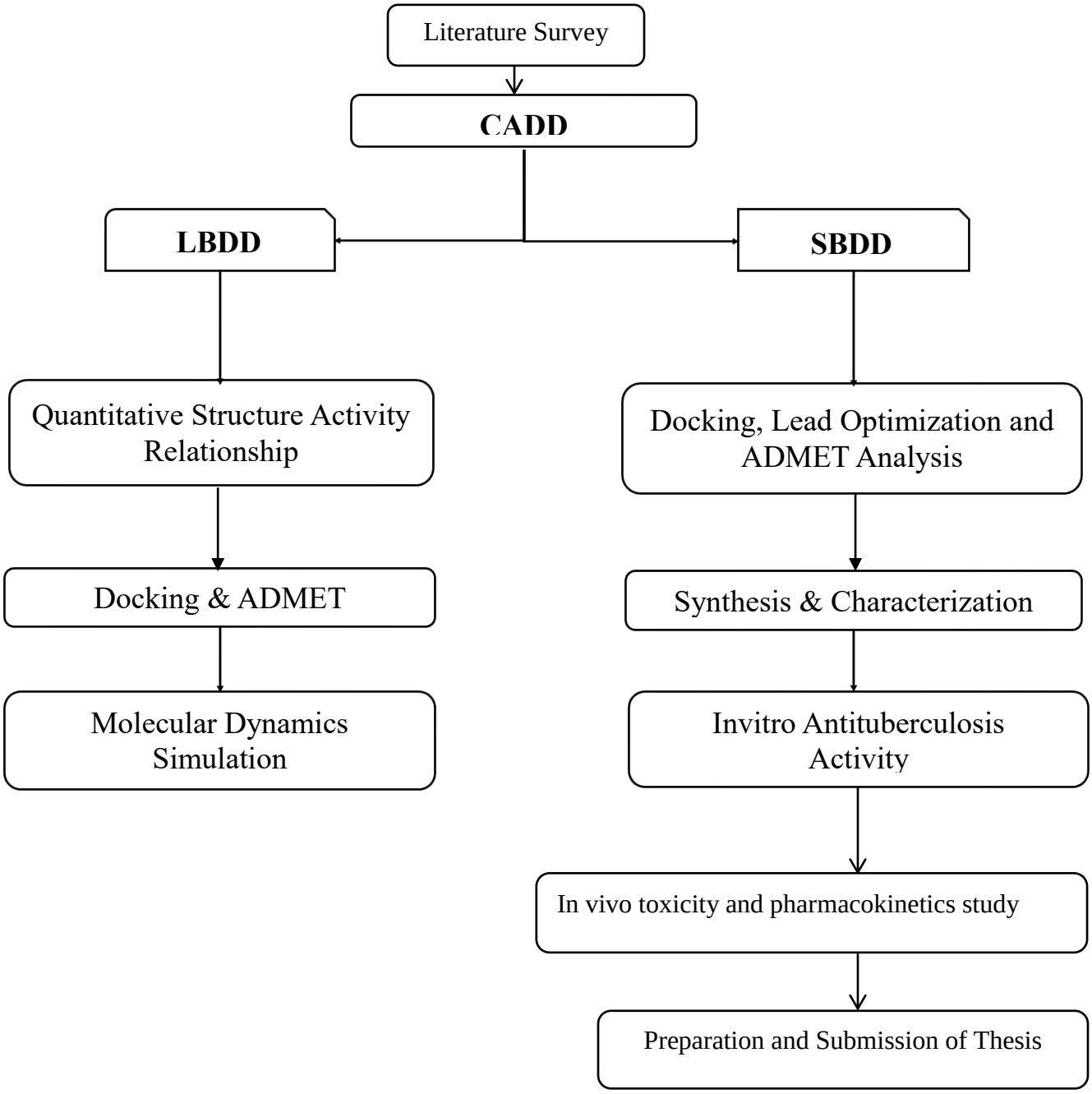


Figure 2: Schematic overview of the work

Docking studies:

Molecules which comes out of best fit from the model will subjected to docking to elucidate the binding affinity and its interaction potential.[20-25] Protein will be downloaded from the protein data bank. The macromolecule used is 5V41 from polyketide synthase XIII. Molecules exhibiting least global minimum will analyzed along with its interactions using Pymol.

Insilico ADME analysis:

Protox II a web server used to rule out various toxicities from the selected compounds.

Docking interactions:

Docking studies will carried out using Autodock vina. Protein will downloaded from the protein data bank. PKS 13 5V41. Proteins and ligands will prepared by adding polar hydrogen aiding Koll man charges and Gasteiger charges. Binding energies will calculated and interactions will be seen from best poses using discovery studio visualiser. Compounds showing best binding affinity with least energy will be selected for synthesis.

Insilico ADME analysis :

Swiss ADME will used to access the various toxicities like carcinogenicity, mutagenicity, hepatotoxicity, for the selected compounds. Then it will also analyzed for Lipinski's rule for drug likeness and bioactivity scores.

Impact of Research Work on academics /industry/ society

Tuberculosis (TB) remains the most frequent and important infectious disease causing morbidity and death. Unfortunately, no new drugs except rifabutin and rifapentine have been marketed for TB in the US and other countries during the 40 years after release of rifampicin. There are a number of constraints that have deterred companies from investing in new anti-TB drugs. The research is expensive, slow and difficult, and requires specialized facilities for handling MTB. There are few animal models that closely mimic the human TB disease. Development time of any anti-TB drug will be long. In fact, clinical trials will require the minimum six-month therapy, with a follow-up period of one year or more. In addition, it is hard to demonstrate obvious benefit of a new anti-TB agents over pre-existing drugs, since clinical trials involve multidrug combination therapy using highly effective ordinary anti-TB drugs. Finally, there is the perceived lack of commercial return to companies engaged in the development of new anti-TB drugs, because over 95% of TB cases worldwide are in developing countries

There are a number of difficulties in drug-design for the development of new drug formulations with increased potential for antimycobacterial effects, excellent pharmacokinetics, and tolerability. In the proposed research work, is to develop highly active, low-cost drugs which can be used not only in industrialized countries but also in developing countries, since the incidences of AIDS-associated intractable TB and MAC infections are rapidly increasing in the latter.

Requirements

- Condenser
- Rbf
- Hot plate
- Heating mantle
- Conical
- Beaker
- Measuring cylinder
- Glass rod
- Magnetic stirred(beads)
- Magnetic retriever
- Funnel

Instrument:

- Vacuums pump
- Rota evaporator
- NMR
- MASS
- IR

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